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Concentrations of airborne culturable bacteria in 100 large US office buildings from the BASE study

Abstract This paper presents summary statistics of airborne culturable bacteria from the US Environmental Protection Agency Building Assessment Survey and Evaluation (BASE) study. Air samples were collected with single-stage, multiple-hole, agar impactors in 100 large office buildings in 1994–1998 to obtain normative data on indoor environmental quality. Bacterial concentrations were compared by incubation temperature, location, season, and climate zone. Forty-one percent of the samples were below the 2- or 5-min detection limits (18 or 7 CFU/m^3, respectively) but less than 1% were overgrown. Mesophilic bacteria (30°C) accounted for >95% of culturable bacteria, both indoors and outdoors. Average concentrations were higher outdoors, except for Gram-positive cocci, which were the only group that were significantly higher indoors (39 vs. 24 CFU/m^3), and Gram-negative cocci, for which both concentrations were low and the difference were not significant. Outdoor concentrations of culturable bacteria were somewhat higher in winter (194 vs.165 CFU/m^3), and the two dominant outdoor groups were unknown bacteria and Gram-positive rods. Conversely, indoor concentrations were significantly higher in summer (116 vs. 87 CFU/m^3), consisting primarily of unknown bacteria and Gram-positive cocci. Bacterial concentrations were within the ranges reported in previous studies of non-problem buildings, and the extreme aggregated indoor concentrations (e.g. the 90th percentile, 175 CFU/m^3) of these 100 representative buildings may serve as upper bounds to develop interpretation guidelines for office environments and similar non-manufacturing workplaces in various climate zones.

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Key words: Bioaerosols; Airborne culturable bacteria; Office buildings; Building Assessment Survey and Evaluation (BASE) study; Baseline exposure assessment.

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Practical Implications
The Building Assessment Survey and Evaluation (BASE) study was one of the most comprehensive investigations of indoor environmental quality in which a standardized protocol was used to measure bioaerosols in 100 typical US office buildings. The information on the indoor and outdoor concentrations of airborne bacteria in different climate zones during the heating and cooling seasons has expanded the baseline data available for interpretation of measurements from building investigations. With suggested refinements, the BASE protocol may serve as a guide for future studies of bioaerosol concentrations, building characteristics, and occupant perceptions of the indoor environment.

Introduction

The US Environmental Protection Agency (USEPA) convened a steering committee of federal and non-federal experts who provided their recommendations for the design, planning, and implementation of a major study of indoor environmental quality (IEQ) in public and commercial buildings – the Building Assessment Survey and Evaluation (BASE) study (http://www.epa.gov/iaq/largebldgs/base/index.html, accessed 22 March 2005). The primary goal of the study was to define the status of the existing building stock with respect to determinants of IEQ and occupant perceptions by collecting normative data on environmental parameters (e.g. temperature and formaldehyde concentration), building characteristics (e.g. age and smoking policy), and occupant perceptions of comfort and IEQ (e.g. self-reported symptoms and descriptions of the work environment) (USEPA, 2003).

In this cross-sectional study conducted between 1994 and 1998, data were collected in 100 large buildings in the continental US stratified into 10 climate zones based on the engineering design conditions for summer and winter temperature (ASHRAE, 1989; Table 1). The number of buildings in each zone was determined

Table 1 Distribution of BASE buildings by climate zone, state, and season

Climate zone	States ($n = 25$)[a]	Number of buildings			
		State total	Summer	Winter	Total by climate zone
A: Cool winter, dry and cool-to-moderate or hot summer	Colorado	3	3	0	6
	Nevada	3	0	3	
B: Cool winter, damp and cool-to-moderate summer	Illinois	3	3	0	23
	Massachusetts	3	0	3	
	Michigan	3	0	3	
	Minnesota	3	0	3	
	New York	6	6	0	
	Pennsylvania	2	2	0	
	South Dakota	3	0	3	
C: Cool winter, damp and hot summer	Missouri	2	2	0	5
	Nebraska	3	0	3	
D: Moderate winter, dry or damp and cool-to-moderate summer	Florida[b]	3	0	3	17
	Georgia	3	3	0	
	Maryland	3	3	0	
	North Carolina	3	0	3	
	South Carolina	2	0	2	
	Tennessee[b]	3	3	0	
E: Moderate winter, dry and hot summer	California[b]	3	1	2	6
	New Mexico	3	3	0	
F: Moderate winter, damp and hot summer	Arizona	3	0	3	13
	Tennessee[b]	3	3	0	
	Texas	7	5	2	
G: Hot winter, dry or damp and cool-to-moderate or hot summer	Florida[b]	4	4	0	7
	Louisiana	3	0	3	
H: Hot winter, dry or damp and hot summer	Arizona	5	2	3	5
I: Moderate winter, damp and cool-to-moderate summer	Oregon	3	3	0	6
	Washington	3	0	3	
J: Hot winter, damp and cool-to-moderate summer	California[b]	12	6	6	12
Total number of buildings		100	52	48	100

[a]The 37 cities in which the buildings were located were not identified to maintain confidentiality.
[b]States with BASE buildings in more than one climate zone (Florida: zones D and G; Tennessee: zones D and F; California: zones E and J).

proportionally by the population size of the zone. Therefore, more buildings were sampled in zones B, D, F, and J. Buildings meeting certain criteria were selected randomly, excluding only buildings with highly publicized IEQ problems so that non-problem and problem buildings were included in proportion to their occurrence in the total building population (Womble et al., 1999). Each building was studied once during a 1-week period in summer or winter following a standardized protocol for environmental measurements and administration of the occupant survey (USEPA, 2003).

The areas surrounding the buildings were described primarily as urban (73%) or suburban (23%) with few large office buildings in rural settings (4%) (Burton et al., 2000). Forty-four buildings had windows that could be opened, of which an average of 72% were reported to be operable (Burton et al., 2000). More buildings reported past water damage (71%) than current water leaks in the occupied space (34%); however, the former may be related to building age (range: 1–147 years; median: 28 years) (Girman et al., 2002).

The authors of this paper participated in neither the design nor implementation of the BASE study, but have summarized and interpreted portions of the data on biological agents, which included air samples (culturable fungi and bacteria as well as total fungal spores but the latter only for 44 buildings from 1996 to 1998) (Macher et al., 2001; Womble et al., 1999), dry and wet bulk samples (culturable fungi and bacteria), and dust samples (culturable fungi and bacteria as well as cat and dust-mite allergens) (Macher et al., 2005). This paper presents summary statistics on the concentrations of culturable bacteria in indoor and outdoor air.

The airborne bacteria to which people are exposed daily seldom cause human illness, although some bacteria are agents of hypersensitivity, infectious, or inflammatory diseases. Endotoxin, a component of the outer membrane of Gram-negative bacteria, has been recognized as a health hazard in various occupations (Myatt and Milton, 2001) and associated with asthma severity (Park et al., 2001). Many bacteria are essential to the earth's ecology and to human health, e.g. Gram-positive and -negative bacteria in soil, water, and on leaf surfaces as well as Gram-positive bacteria on human skin and scalp and Gram-negative intestinal bacteria. Studies have shown that the bacteria found in indoor air generally were shed by building occupants or entered with outdoor supply air and that the risk of

illness from environmental bacteria increases when they enter buildings in inappropriate numbers or multiply indoors (Otten and Burge, 1999). Sampling for culturable bacteria generally underestimates human exposure because non-culturable cells (often a large fraction of total bacteria) are not detected (Toivola et al., 2002). However, this method is widely used to assess IEQ, and information on baseline concentrations of culturable bacteria in representative office environments is essential for proper interpretation of measurements made to investigate problem buildings.

Methods

Sample collection

Air samples for culturable bacteria were collected on tryptic soy agar (TSA) using four, single-stage, multiple-hole, agar impactors (N-6 sampler; d_{50}: 0.6 μm; flow rate: 28.3 l/min; Thermo Andersen, Franklin, MA, USA). Before each round of sampling, the impactors were cleaned with cotton swabs wetted with isopropyl alcohol (USEPA, 2000). Airflow rate was measured in the field with a calibrated rotameter in-line between the sampler and the vacuum pump (USEPA, 2003). To ensure data quality, duplicate samples and shipping and field blanks were collected at each building.

Samples were collected either in summer (June to September, 52 buildings) or winter (December to April, 48 buildings) on the third day (Wednesday) of the 1-week visit, in the morning (9 AM to 1 PM) and repeated in the afternoon (12:30 to 5:30 PM), at three, randomly selected, indoor sites (F1, F3, and F5) and one outdoor location (F0, near the air intake of the air-handling unit serving the indoor test space) (USEPA, 2003). These locations were designated 'Fixed' sites at which time-integrated samples were collected in contrast to the 'Mobile' sites (M1–M5) at which continuous, indoor measurements were made. Because sampling sequence was neither random nor completely systematic, the time interval between site-specific AM and PM samples varied widely (1 to >6 h), especially for outdoor samples which were collected sequentially at mid-day or first in the morning and last in the evening. Therefore, concentrations for the morning and afternoon sampling periods were not compared.

At each building, approximately 48 air samples were collected for bacterial measurements: (two sampling durations: 2-/5-min) × (two sampling periods: AM/PM) × (two incubation temperatures: 30°/55°C) × [six sets of samples: (three indoor sites + one indoor duplicate) + (one outdoor site + one outdoor duplicate)]. Air samples for the two incubation temperatures and sampling durations were collected simultaneously at the indoor sites without duplicate samples: two of the impactors were stopped after 2 min and the other two were operated an additional 3 min. Otherwise, duplicate samples of the same sampling duration were collected simultaneously, first by sampling for 2 min onto four plates (two for each incubation temperature) followed by replacement of the media and re-sampling for 5 min.

Samples were shipped overnight to the analytical laboratory where they were incubated in the dark at 30 ± 2°C for a minimum of 3 days or at 55 ± 2°C for a minimum of 7 days (USEPA, 2003). Seven bacterial groups were reported for the 30°C samples based on Gram stain reaction (positive or negative), cell shape (coccus or rod), and distinguishable type of Gram-positive rod (actinomycetes and *Bacillus* species), plus unknown isolates (i.e. those that could not be classified with sufficient confidence in one of the former categories). Only actinomycetes, *Bacillus* species, and unknown bacteria were reported for the 55°C samples (USEPA, 2003). Culture results were reported as the number of colony-forming units (CFUs) for each bacterial group per sample (with positive-hole correction) and adjusted by sample volume to obtain bacterial air concentration (CFU/m^3). For our analyses, the three groups of Gram-positive rods were reported separately and summed for the total concentration of Gram-positive rods, and the seven bacterial groups from both incubation temperatures were reported separately and summed for total bacterial concentrations.

A total of 5201 bacterial samples were collected, 419 blank samples (8.1%) and 4782 air samples (91.9%; Table 2), including 1593 duplicate air samples (30.6%). By design, similar numbers of duplicate samples were collected outdoors and indoors (15.2 and 15.4%). However, more duplicate samples were collected at indoor sites F5 and F1 than F3 (7.4 and 5.2% vs. 2.8%) because early versions of the protocol specified that duplicate samples be collected at these sites. The final protocol permitted use of any indoor site to accommodate physical restrictions and other limitations at the buildings (USEPA, 2003).

Blank samples were reported as not analyzed, not reported, or below the detection limit of 1 CFU per culture plate for all but two samples (<0.5%) for which single colonies of unknown bacteria were reported. Approximately 41% of non-blank samples were below the respective detection limits: 44 and 38% of 2- and 5-min samples; 43 and 36% of indoor and outdoor samples; and 3 and 78% of 30°C and 55°C samples, respectively (Table 2). Few samples (<1%) were lost because overgrown. However, more outdoor than indoor samples were overgrown (2.2% vs. 0.1%) as were more of the 30 °C than 55 °C samples (1.6% vs. 0) and a slightly higher fraction of 5- than 2-min samples (1.0% vs. 0.5%; Table 2).

The 2- and 5-min samples produced similar concentration estimates (average: 74 and 57 CFU/m^3; s.d.:

Table 2 Number of air samples by sampling duration, sampling location, and incubation temperature

	Total air samples		Sampling duration[a]				Sampling location[b]				Incubation temperature			
			2-min		5-min		Indoors		Outdoors		Mesophilic bacteria[c]		Thermophilic bacteria	
Data entry	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Not analyzed/pump failure	62	1.3	33	1.4	29	1.2	46	1.4	16	1.0	56	2.3	6	0.2
Overgrown	38	0.9	13	0.5	25	1.0	4	0.1	34	2.2	38	1.6	0	0
Below detection limit	1941	40.6	1037	43.5	901	37.7	1365	42.7	576	36.4	68	2.8	1873	78.3
Above detection limit	2741	57.3	1303	54.6	1435	60.0	1784	55.8	956	60.4	2227	93.2	514	21.5
Total number of samples	4782		2386		2390		3199		1582		2389		2393	

[a]Six samples with sampling time of 3 or 3.3 min were not included; three of these samples were below and three samples were above the respective detection limits.
[b]One above-detection sample for which sampling site identification was missing was not included.
[c]Fifty-one samples that mistakenly were incubated at 23°C rather than 30°C were included in this group.

135 and 101 CFU/m^3; minimum: both below the detection limit; median: 18 and 14 CFU/m^3; maximum: 1820 and 1286 CFU/m^3, respectively). As expected, average and maximum colony counts were approximately twice as high for the longer sampling time (2-min samples: 4 and 103 CFU/plate; 5-min samples: 8 and 182 CFU/plate, respectively). Few samples met or exceeded a total colony count of 25 CFU/plate, a recommended minimum plate count for reliable estimation of the concentration of culturable bacteria in water or food samples (2.4 and 8.8% for 2- and 5-min samples, respectively) (APHA, 1998, 2001). However, 13.6 and 28.1% of the samples, respectively, equaled or exceeded 10 CFU/plate, a minimum count suggested for air samples (ACGIH, 1999).

Data analysis

The USEPA supplied an electronic version of the BASE data in May 2002, which was evaluated for the consistency of the entries and then transposed to calculate aggregated air concentrations. For this analysis, questionable entries (e.g. count and concentration entries disagreed) either were modified using professional judgment (e.g. 'not reported' was changed to 'not detected') or excluded (e.g. overgrown samples or pump failures). Individual samples with concentrations below the respective detection limits (2-min samples: 18 CFU/m^3; 5-min samples: 7 CFU/m^3) were set to zero to avoid overestimation of the aggregated concentrations when the seven bacterial groups and 16 outdoor and 32 indoor samples were combined for each building.

Data analysis was conducted using SAS version 8.2 (SAS Institute, Cary, NC, USA). Both raw and natural logarithm-transformed values were tested for a normal distribution. Separate analyses were conducted for each location (outdoor samples, site-specific indoor samples, and building-wide average indoor concentrations), bacterial group (total bacteria and the three most prevalent identifiable groups: actinomycetes and *Bacillus* species in outdoor air and Gram-positive cocci in indoor air), incubation temperature (30°C and 55°C), and sampling duration (2-min and 5-min samples). None of the data (i.e. neither the total nor any of the groups, overall or for either incubation temperature or sampling time) could be described by either a normal or lognormal distribution. Therefore, geometric means and geometric standard deviations were not reported.

The precision of duplicate samples was evaluated by calculating the relative percent difference (RPD).* The average RPD for 1536 paired primary and duplicate samples was 0.57 (median = 0.25; mode = 0; maximum = 2, which occurred when one sample was below the detection limit and the other had a positive concentration – 48 pairs for 30°C samples and 190 pairs for 55°C samples). Mesophilic and thermophilic samples had similar average RPDs (0.59 and 0.54, respectively), but the 30°C samples showed much higher variation between paired samples than did the 55°C samples (the averages of the absolute values of the concentration differences were 61.5 and 7.4 CFU/m^3, respectively), primarily because mesophilic bacteria were isolated more often at higher concentrations than thermophilic bacteria. Primary and duplicate 2-min and 5-min samples had similar average RPDs and also similar standard deviations (0.56 and 0.57, 0.72 and 0.71, respectively). A preliminary comparison of using either primary samples alone, the average of primary and duplicate samples, or the higher of the pair led to the choice of the average concentration as the best estimate of bacterial air concentration for sites with duplicate samples (data not shown).

To obtain the best concentration estimates for each building, the multiple air samples were aggregated into one indoor and one outdoor concentration in the following steps. (i) Average the primary and duplicate

*RPD = $\frac{|a1-a2|}{\frac{a1+a2}{2}}$, where $a1$ and $a2$ were the concentrations of co-located primary and duplicate samples.

samples if there was a duplicate. (ii) Average the 2-min and 5-min samples by taking volume-weighted averages. (iii) Average the morning and afternoon samples. (iv) Report separately the concentrations of thermophilic and mesophilic bacteria and also sum their concentrations by location for the one outdoor and three indoor sites at each building. (v) Average the concentrations from the three indoor sites. The minimum detection limits for the 100 aggregated indoor and outdoor samples were 0.8 and 2.5 CFU/m^3, respectively, if no samples were missing.

Indoor–outdoor ratios were calculated using the final aggregated measurements for either 300 site-specific indoor concentrations [sites F1, F3, and F5 separately; step (iv)] or 100 building-wide average indoor concentrations [step (v)] vs. 100 corresponding outdoor concentrations. A nonparametric Kruskal–Wallis test was used to compare the aggregated concentrations by location (building-wide average indoor vs. outdoor) and season (summer vs. winter). Regression and correlation analyses were performed on the aggregated concentrations of culturable airborne bacteria to evaluate overall patterns between the indoor and outdoor environments and among the indoor sampling sites.

Results

Aggregated concentrations of the seven bacterial groups in 100 buildings are summarized in Table 3 by location and season; and concentrations for the five main bacterial groups are summarized in Table 4 by location and incubation temperature. Minimum aggregated air concentrations generally were below the respective detection limits. Outdoor bacterial concentrations were higher in winter (194 vs. 165 CFU/m^3), although the differences were not statistically significant, while indoor concentrations were significantly higher in summer (116 vs. 87 CFU/m^3, $P < 0.001$; Table 3). Increased concentrations of unknown bacteria and Gram-positive rods contributed to the higher winter bacterial concentration outdoors, whereas elevated unknown bacteria and Gram-positive cocci contributed to the higher summer concentration indoors. A significant seasonal difference was observed indoors for Gram-positive cocci (summer: 48 CFU/m^3; winter: 29 CFU/m^3; $P < 0.001$), which was the only group for which the mean concentration was higher indoors than outdoors in both seasons (Table 3).

Average concentrations of total bacteria (combining seasons and incubation temperatures) were significantly higher outdoors (179 vs. 102 CFU/m^3, $P < 0.01$; Table 4a). The 50th, 75th and 90th percen-

Table 3 Comparison of average aggregated concentrations of airborne culturable bacteria (sum of meso- and thermophilic bacteria) (CFU/m^3) by sampling location and season ($n = 100$ buildings; summer: $n = 52$ buildings; winter: $n = 48$ buildings)

	Indoor samples		Outdoor samples	
Bacterial group	Summer	Winter	Summer	Winter
Total Gram+ rods	10.6	11.4	33.6	43.6
(Actinomycetes)	(2.0)	(1.2)	(6.4)	(3.4)
(*Bacillus* species)	(6.9)	(6.6)	(19.9)	(23.4)
(Other Gram+ rods)	(1.7)	(3.5)	(7.3)	(16.9)
Gram+ cocci	48.3*	28.7*	26.2	21.8
Gram− rods	3.5	2.6	14.9	11.0
Gram− cocci	1.6	1.3	1.1	3.3
Unknown	51.8	42.6	89.1	114.7
Total bacteria	116.0*	86.7*	165.0	194.5

*$P < 0.001$ (higher summer vs. winter indoor bacterial air concentrations using Kruskal–Wallis test).

Table 4 Aggregated concentrations of airborne culturable bacteria (CFU/m^3) by sampling location and incubation temperature (summer and winter data were combined and only total Gram-positive rods were reported) ($n = 100$ buildings except for Table 4b where indoor $n = 98$ and outdoor $n = 97$ buildings)

Sampling location	Bacterial group	Average	s.d.	Maximum	Median
(a) Total bacteria[a] (sum of mesophilic and thermophilic bacteria)					
Indoors	Gram+ rods	11.0*	10.7	67.1	7.8
	Gram+ cocci	38.9**	27.4	146.4	32.5
	Gram− rods	3.0*	5.2	38.0	1.7
	Gram− cocci	1.5	5.1	30.1	<0.8
	Unknown	47.4*	41.6	206.2	38.2
	Total bacteria	101.9*	54.7	281.1	91.4
Outdoors	Gram+ rods	38.4*	55.9	353.6	19.3
	Gram+ cocci	24.1**	36.4	203.6	10.3
	Gram− rods	13.0*	32.0	183.5	2.6
	Gram− cocci	2.2	8.0	66.5	<2.5
	Unknown	101.4*	111.3	754.1	62.6
	Total bacteria	179.2*	168.2	958.8	121.2
(b) Mesophilic bacteria[b]					
Indoors	Gram+ rods	9.2*	8.7	43.2	6.6
	Gram+ cocci	39.7**	27.1	146.4	32.8
	Gram− rods	3.1*	5.2	38.0	1.7
	Gram− cocci	1.5	5.2	30.1	<0.8
	Unknown	46.7*	40.2	205.4	38.2
	Total bacteria	100.3*	49.6	275.5	91.4
Outdoors	Gram+ rods	31.6*	48.5	351.1	16.5
	Gram+ cocci	24.9**	36.7	203.6	11.8
	Gram− rods	13.4*	32.4	183.5	3.7
	Gram− cocci	2.2	8.1	66.5	<2.5
	Unknown	101.5*	109.8	754.1	62
	Total bacteria	173.7*	158.2	943.7	121.1
(c) Thermophilic bacteria[a]					
Indoors	Gram+ rods	2.0*	3.4	23.9	0.8
	Unknown	1.7*	7.1	59.3	<0.8
	Total bacteria	3.6*	8.0	60.1	1.2
Outdoors	Gram+ rods	7.8*	16.9	94.6	2.5
	Unknown	2.9*	7.0	54.4	<2.5
	Total bacteria	10.7*	22.3	149.0	3.8

*$P < 0.05$ (higher outdoor concentration using Kruskal–Wallis test); within the group Gram+ rods, the three subgroups (actinomycetes, *Bacillus* species, and other Gram+ rods) individually also were significantly higher outdoors than indoors in Table 4a,b as were the two subgroups (actinomycetes and *Bacillus* species) in Table 4c.

**$P < 0.001$ (higher indoor concentration using Kruskal–Wallis test).

[a]All minimum concentrations were below the respective detection limits.

[b]Minimum concentrations were below the respective detection limits, except for indoor Gram+ cocci (1.3 CFU/m^3), indoor unknown bacteria (2.5 CFU/m^3), total indoor bacteria (17.6 CFU/m^3), and total outdoor bacteria (6.3 CFU/m^3). Some categories had fewer than 100 buildings because of exclusion of questionable data (e.g. samples lost because of pump failure, Table 2).

tiles of total bacteria were 91, 124, and 175 CFU/m^3 for indoor aggregated samples and 121, 134, and 370 CFU/m^3 for outdoor aggregate samples. Comparisons within each bacterial group showed that concentrations were significantly higher outdoors for Gram-positive and -negative rods and unknown bacteria (Table 4) but were significantly higher indoors for Gram-positive cocci (39 vs. 24 CFU/m^3, $P < 0.001$). Gram-negative cocci also were higher outdoors, but the concentrations were the lowest of any group and the differences were not significant. Figure 1 shows the overall composition of culturable bacteria in indoor and outdoor air (Table 4a).

More bacteria grew at 30°C in all groups and both locations (Table 4b,c) as well as both seasons (data not shown). Therefore, the concentrations of mesophilic bacteria were similar to those for total bacteria (Table 4a,b). Both indoors and outdoors, unknown bacteria accounted for the highest proportion of mesophilic bacteria (Table 4b) followed by Gram-positive cocci indoors and Gram-positive rods outdoors. Only *Bacillus* species, actinomycetes, and unknown bacteria were identified for the samples incubated at 55°C; although 'other Gram-positive rods' also were reported for three samples. The outdoor concentration of thermophilic Gram-positive rods was approximately three times higher than the outdoor concentration of unknown isolates (7.8 vs. 2.9 CFU/m^3), but the indoor concentrations were lower and approximately equal (2.0 vs. 1.7 CFU/m^3; Table 4c).

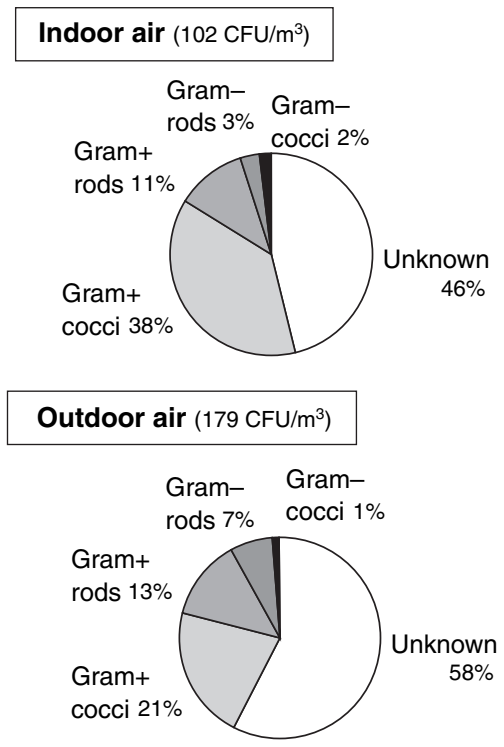

Fig. 1 Composition of culturable bacteria in indoor and outdoor air in 100 large office buildings

The average (and maximum; data not shown) concentrations of total culturable bacteria were higher outdoors in all climate zones when data from both seasons were combined (Table 5). Stratification by season showed that this pattern was observed in winter in all ten zones and in summer in all but two zones, in which indoor concentrations were slightly higher than outdoor concentrations (zone C: 93 vs. 87 CFU/m^3; and zone F: 118 vs. 102 CFU/m^3, respectively; Table 5). Comparing seasonal bacterial concentrations for each location, outdoor averages were higher in winter than summer in all but zones A and B, although the differences were small in zones D and J ($\leq +10$ CFU/m^3). However, average indoor concentrations were higher in summer in five zones (zones A, B, D, G, and H; 58 buildings) but higher in winter in the other five zones (zones C, E, F, I, and J; 42 buildings; although the difference was small for zone F, $+4$ CFU/m^3). Overall across the ten climate zones, the concentration of culturable bacteria in outdoor air showed a consistent, although not statistically significant, seasonal pattern (higher in winter) although different numbers of buildings were studied in each region (range: 5–23 buildings per zone) and summer and winter conditions vary widely across the US (Table 1). Indoor bacterial air concentrations showed a mixed pattern, suggesting that factors other than seasonal differences may have affected bacterial air concentrations at some buildings.

Indoor–outdoor ratios for aggregated building-wide bacterial concentrations ranged from 0.1 to 16.4 (average: 1.3; s.d.: 2.0) and were similar for all three indoor sites (Table 6). Sixty-five buildings had ratios below or equal to 1 (average: 0.5; s.d.: 0.2). Thirty-five buildings had ratios above 1 (average: 2.9; s.d.: 2.9), suggesting the presence of indoor sources in these buildings. Average ratios also were similar when the site-specific ratios were considered separately, i.e. indoor concentrations approximately three times higher than outdoor levels for buildings with ratios > 1 and indoor concentrations

Table 5 Average aggregated concentrations of total airborne culturable bacteria (CFU/m^3) by climate zone, sampling location, and season (see Table 1 for the number of buildings in each climate zone by season)

	Indoors			Outdoors		
Climate zone	Combined seasons	Summer	Winter	Combined seasons	Summer	Winter
A	113	139	88	198	215	180
B	90	125	59	141	181	104
C	99	93	103	186	87	252
D	98	112	81	121	121	122
E	131	120	154	409	329	568
F	120	118	122	178	102	300
G	116	156	62	204	181	235
H	102	136	79	330	278	365
I	97	86	108	133	122	145
J	86	78	94	154	149	159

Table 6 Comparison of indoor–outdoor ratios of aggregated bacterial concentrations

	Indoor–outdoor ratio ($n = 98$)[a]				Number of buildings with indoor–outdoor ratios >1 ($n = 99$)[b]
	Average	s.d.	Maximum	Minimum	
Building-wide indoor average vs. outdoor	1.3	2.0	16.4	0.10	35
Site F1 vs. outdoor	1.3	2.2	19.2	0.06	34
Site F3 vs. outdoor	1.4	2.4	18.4	0.10	37
Site F5 vs. outdoor	1.2	1.8	11.8	0.04	29

[a]The calculations of averages, s.d., minima, and maxima for the indoor–outdoor ratios excluded the two buildings for which all outdoor air samples were below the detection limit.

[b]One building in Texas (for which all indoor and outdoor air samples were below the detection limit) was excluded in the count of the number of pairs, but one building in Illinois (for which all outdoor samples were below the detection limit but the average indoor concentration was positive) was included.

less than half the outdoor levels for buildings with ratios ≤ 1 (data not shown).

Bacterial concentrations at the three indoor sites were moderately but significantly correlated (Pearson correlation coefficients, $r = 0.34$–0.53, $P < 0.001$). However, the outdoor concentration was significantly associated only with site F1 ($r = 0.26$, $P < 0.01$). This association may be due to chance because the indoor sites were chosen randomly. However, an examination of sampling sequence showed that site F1 was sampled immediately after the mid-day outdoor samples more often than the other two indoor sites. Additional correlation analyses showed that indoor samples collected closer in time to the outdoor samples correlated better with ambient measurements, especially for the buildings with lower indoor or equal indoor–outdoor concentrations (data not shown). This finding suggests that temporal variation may complicate the assessment of indoor–outdoor relationships if samples are not collected simultaneously.

When study buildings were divided by indoor–outdoor ratios >1 and ≤ 1, the three indoor sites were significantly correlated with each other ($r = 0.34$–0.57, $P < 0.05$) in both subgroups except for sites F1 and F5 which did not correlate significantly in the first group of buildings. The latter finding again may be due to chance or related to non-random sampling sequence because sites F1 and F5 more often were sampled near times of possibly increased activity related to interrupting work for a mid-day break. For the 35 buildings with higher indoor concentrations, the correlations between indoor and outdoor measurements were higher and outdoor concentrations were significantly associated with both sites F1 and F3 ($r = 0.50$–0.71, $P < 0.01$). For the 65 buildings with lower indoor or equal indoor–outdoor concentrations, the outdoor concentrations were significantly associated with all three indoor sites ($r = 0.47$–0.49, $P < 0.001$). Regression analysis using outdoor concentrations to predict building-wide indoor average concentrations showed that:

1 For buildings with higher indoor concentrations ($n = 35$): Building-wide average indoor concentration = **68 + 0.91** (outdoor concentration), $R^2 = 0.39$.
2 For buildings with lower indoor or equal indoor–outdoor concentrations ($n = 65$): Building-wide average indoor concentration = **54 + 0.14** (outdoor concentration), $R^2 = 0.38$.

The bold values were statistically significant, i.e. the intercepts and regression coefficients were not zero.

These results implied, given the moderate R^2, that in both subgroups of buildings, indoor sources of bacteria were present (i.e. the intercepts exceeded zero: 68 and 54 CFU/m^3 for buildings with indoor–outdoor ratios >1 and ≤ 1, respectively) and that a portion of the bacteria in the indoor air could be attributed to the entry of bacteria from outdoors (i.e. the beta coefficients: 91 and 14%, respectively). Thus existing indoor sources were stronger (+14 CFU/m^3) and penetration of outdoor bacteria was greater (6.5 times) for buildings with higher indoor than outdoor concentrations relative to buildings with lower indoor or equal indoor–outdoor concentrations.

Discussion

The standardized protocol of the BASE study has provided the most comprehensive data available to date on bioaerosol concentrations in large US office buildings. Given the lack of consensus, health-based, numeric standards for interpretation of measurements of indoor airborne bacteria, policy makers and researchers may use data from the BASE study (Table 3–5) in conjunction with findings from other investigations (Bholah and Subratty, 2002; Burton et al., 2000; Dacarro et al., 2000; Górny and Dutkiewicz, 2002; Lis et al., 1997; Reponen et al., 1992; Reynolds et al., 2001; Sessa et al., 2002) as scientific evidence to identify acceptable bacterial concentrations in office settings and to develop and test specific hypotheses. For example, the extreme aggregated indoor bacterial air concentrations identified in the BASE buildings (the 75th or 90th percentiles; 124 and 175 CFU/m^3, respectively) may serve as upper bounds of typical indoor concentrations in offices and similar non-manufacturing workplaces. The BASE study included information for different climate zones in the heating and cooling seasons so that other study results may be compared with normative data collected in the same season from a region with similar heating and cooling requirements.

It is generally assumed that environmental measurements display either normal or lognormal distributions. However, little is known about distributions for

bioaerosol measurements, especially indoor samples (Eudey et al., 1995; Luoma and Batterman, 2000; Spicer and Gangloff, 2003), because of the limited number of samples collected in previous studies or failure to identify the proper data distribution prior to data summary and interpretation. Sofuoglu and Moschandreas (2003) reported that the data for airborne bacteria from the first 41 BASE buildings (1994–1996) were log-normally distributed, but the authors did not describe how they reached this conclusion and it was not replicated in the current analyses for the complete data on 100 buildings. Therefore, further analyses should be conducted to determine the proper distribution and appropriate statistical models for the bioaerosol data from the BASE study.

The large RPDs for duplicate samples in the BASE buildings indicated that the concentrations of culturable airborne bacteria were highly variable, even for samples collected simultaneously at the same site. For non-biological agents for which a homogeneous distribution is assumed (e.g. formaldehyde and airborne particulate matter), large RPDs (e.g. $>30\%$ difference) may reflect sampling problems (e.g. inconsistent equipment performance). However differences between duplicate samples for biological agents may be real and can provide estimates of both sampling and random ('chance') errors. Assuming that the identical impactors used to collect simultaneous samples of airborne bacteria performed comparably, the differences between duplicate samples can be attributed primarily to random variation in bacterial concentrations rather than instrument bias. Investigators have observed differences in air concentrations of culturable microorganisms over time and space of three to four orders of magnitude (AIHA, 1996), even greater than what was observed in the BASE buildings, which were within one order of magnitude for aggregated concentrations and three orders of magnitude for individual samples.

The three site-specific aggregated indoor concentrations were significantly correlated with correlation coefficients ranging from 0.3 to 0.6, but the absolute concentrations varied within and between the sampling sites. This finding indicates that multiple indoor sites may share common indoor and outdoor sources as well as ventilation patterns, resulting in similar magnitudes and fluctuations in bacterial concentrations, but that at individual sites within a building, the concentrations may be higher or lower than elsewhere because of localized sources or other site-specific factors.

The indoor sampling sites should have had no systematic differences, although the estimation of the bacterial air concentration should have been more accurate for sites sampled in duplicate. However, the sequence of sample collection in the morning and afternoon was neither random nor entirely systematic. Most of the BASE buildings ($n = 98$) had mechanical ventilation systems and the indoor sites usually were on the same or adjacent floors that were served by no more than two air-handling systems (Burton et al., 2000). That the indoor–outdoor correlation results were significant for only site F1 for all 100 buildings and for only sites F1 and F3 for the 35 buildings with higher indoor concentrations suggests that the presence of indoor bacterial sources or other site-specific factors may play a greater role in determining indoor bacterial air concentrations than the contribution from outdoor supply air. Possible indoor sources of bacteria are high occupant density or local microbial growth, and possible site-specific factors are different activity levels near the time of sample collection. These findings also emphasize the importance of sampling at multiple locations within a building to characterize exposure more accurately, given the lack of affordable personal bioaerosol samplers.

The large number (approximately 50%) of isolates that could not be identified (the unknown group; Figure 1) illustrates one of the limitations of relying on culture-based methods alone, identification solely by a simple Gram stain, and failure to subculture isolates that initially cannot be identified. Measuring only culturable bacteria also underestimates exposure, e.g. Toivola et al. (2002) reported a concentration ratio of viable to total bacteria of close to 1:100. Nevertheless, many previous studies have relied on the culture method and it continues to be the most widely available means of measuring indoor bacterial contamination and distinguishing bacteria of human and environmental origin (Otten and Burge, 1999).

In the BASE dataset, over 75% of the individual samples incubated at 55°C were below the 2- and 5-min detection limits (Table 2; 83% and 69% of indoor and outdoor air samples, respectively, data not shown), indicating that the concentrations of thermophilic bacteria typically were low. Therefore if resources are limited, a larger sample volume (>0.14 m^3) for thermophilic bacteria or incubation of samples only at a moderate temperature (30°C) may be recommended for non-problem building investigations.

Similar fractions of 2-min and 5-min samples yielded measurable results (55 and 60%) or were overgrown (0.5 and 1.0%; Table 2). The two sets of measurements also produced similar concentration estimates (average: 74 vs. 57 CFU/m^3), and the means and standard deviations of the RPDs for duplicate samples were similar for both sampling durations. However, the average colony counts were twice as high for 5-min samples (8 vs. 4 CFU/plate) indicating that they provided more reliable estimates of bacterial air concentrations and that collection of a larger sample volume (0.14 vs. 0.06 m^3 of air) may be more appropriate for non-problem building investigations. In fact, sampling time could be extended because few (1%) of the 5-min plates were overgrown, no growth was seen

on 38% of them (Table 2), and only slightly more than a quarter (28%) of them yielded ≥10 CFU/plate, a suggested minimum plate count for reliable and representative measurement of the concentration of culturable airborne bacteria (ACGIH, 1999).

For this paper, we aggregated approximately 16 outdoor and 32 indoor measurements assuming that samples for two sampling durations at two times per day, and from three randomly selected indoor locations provided the single best concentration estimates with which to compare locations, seasons, and climate zones. The aggregated indoor values also could be assumed to provide reasonable estimates of exposures for an 8-h workday during the week for which the occupants reported symptoms and perceptions of IEQ. Integration of multiple samples collected throughout the day and in different locations within a large building may better characterize indoor bioaerosol concentrations than samples from only one location, at least on a relative scale. However, the occupants of the buildings in the BASE study may not have spent their entire workdays near one of the indoor sampling sites, and the questionnaires were administered the day following collection of bioaerosol samples. Therefore, associations between measurements of culturable airborne bacteria and self-reported symptoms may not be strong in these subjects even if some of the occupants' symptoms were related to indoor bioaerosol exposures.

Temperature, available moisture, and hours of daylight cause seasonal variation in outdoor bioaerosol concentrations. Several studies have found that culturable bacteria are more prevalent in summer than winter in some regions because of warm, dry, dusty conditions and higher agricultural or human activities in summer in contrast to cool wet conditions with possible snow cover in winter (Bovallius et al., 1978; Di Giorgio et al., 1996; Jones and Cookson, 1983; Kelly and Pady, 1954; Tong and Lighthart, 2000). Endotoxin from Gram-negative bacteria also has been found to be higher outdoors in spring and summer than in winter in some regions (Carty et al., 2003; Park et al., 2000). However, no statistically significant seasonal variation in the outdoor concentration of total bacteria or Gram-negative bacteria was observed at the 100 BASE buildings. While there was no significant seasonal difference in the indoor concentrations of culturable Gram-negative bacteria either, the aggregated concentration of total bacteria was significantly higher indoors in summer (Table 3).

Seasonal comparisons of the BASE data can be made only in a general sense, because no buildings were studied in both seasons. Therefore, we cannot conclude directly that bacterial concentrations were higher or lower in either season. To determine the seasonal variation of bioaerosol concentrations, the same buildings should be studied in the heating and cooling seasons, which vary regionally. Given the strong effect of season on outdoor bacterial reservoirs and the results of the regression analysis, the BASE study confirmed the importance of sampling the outdoor supply air to estimate the potential contribution of ambient sources to indoor bioaerosol concentrations. Outdoor measurements did not always predict indoor concentrations, but information on ambient levels may help investigators recognize if indoor concentrations are elevated because of indoor sources, e.g. biological contamination, a high density of occupants, or increased occupant activity.

Indoor and outdoor measurements should be made concurrently, if possible, to minimize differences because of temporal variability. Outdoor samples collected simultaneously with indoor samples may show better correlation with indoor measurements than was seen in this study in which the outdoor samples were collected before or after the indoor samples. More representative timing of outdoor measurements (e.g. mid-morning and mid-afternoon) would allow examination of possible diurnal fluctuations, which may be caused by changes in air and surface temperatures as well as the moisture content of outdoor air and by fluctuations in wind speed and turbulence.

Conclusion and implications

The summary data in this paper provide baseline concentrations of airborne culturable bacteria in representative large office buildings across the continental US. Collection of samples both indoors and outdoors allowed estimation of the contributions of building-related sources and ambient air. However, simultaneous measurements of indoor and ambient bioaerosol concentrations would have provided better information on the relative contributions of indoor and outdoor sources. The high variability between duplicate samples and among three indoor sampling sites suggests that multiple samples are required to capture the temporal and spatial variation of bacterial air concentrations in large office buildings. Likewise, convenient and affordable personal samplers are needed to better characterize inhalation exposures for epidemiological studies seeking to understand the associations between bioaerosols and health effects (Institute of Medicine, 2004). Mesophilic bacteria comprised a much larger proportion of total culturable bacteria than thermophiles in the BASE study as in other investigations of residential and office environments (Reynolds et al., 2001), and measurement of only mesophiles may suffice in future studies of non-complaint buildings. A sampling time of 5 min (air volume: 0.14 m^3) provided a more reliable estimate of air concentration than did a shorter sampling time, and an even larger sample volume may be appropriate for measurement of culturable bacteria in non-complaint building investigations.

Aggregated bacterial concentrations in the 100 office buildings in the BASE study were not high (indoor median and maximum: 91 and 281 CFU/m^3, respectively) and were in the ranges reported in other studies of non-complaint office buildings in the US and around the world (Bholah and Subratty, 2002; Dacarro et al., 2000; Lis et al., 1997; Parat et al., 1997; Reynolds et al., 2001; Sessa et al., 2002). Outdoor concentrations of airborne bacteria generally were higher than indoor concentrations (outdoor median and maximum: 121 and 959 CFU/m^3, respectively) with opposite seasonal patterns, i.e. increased outdoor concentrations (approximately +18%) and decreased indoor concentrations (approximately −25%) in winter compared with summer.

The concentration of culturable bacteria in outdoor air showed a consistent seasonal pattern across ten climate zones, and bacteria associated with soil and plant surfaces (e.g. Gram-positive rods) were more abundant in outdoor air nationwide. Indoor bacterial concentrations showed more seasonal and regional differences mainly because of a significantly higher concentration of Gram-positive cocci in summer, which may reflect changes in occupant dress and activities as well as ventilation patterns between the heating and cooling seasons. In buildings with indoor–outdoor concentration ratios >1, indoor estimates of the concentration of airborne culturable bacteria attributable to indoor sources were slightly higher and associations with outdoor concentrations were stronger than in buildings with ratios ≤ 1 suggesting the existence of stronger indoor sources and substantially greater infiltration of outdoor air in the 35 buildings with ratios above one. The protocol used in the BASE study already has been adopted for other investigations (Chao et al., 2003; Crandall and Sieber, 1996; Reynolds et al., 2001; Schillinger et al., 1999), and the lessons learned from examinations of the BASE results will provide valuable insight for future studies of IEQ in office buildings and the development of consensus investigation protocols and evaluation guidelines.

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